Second Edition

CULTURE OF ANIMAL CELLS

A Manual of Basic Technique

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Alan R. Liss, Inc., New York

Cover Illustrations. *From the top: Vero cells growing on microcarriers; suspension culture vessels; primary explant from human mammary carcinoma; human glioma cells.*

Address all Inquiries to the Publisher
Alan R. Liss, Inc., 41 East 11th Street, New York, NY 10003

Printed in the United States of America

First Edition published 1983
Second Printing, April 1984
Third Printing, November 1984

Second Edition published 1987

Library of Congress Cataloging-in-Publication Data

Freshney, R. Ian.
Culture of animal cells.

Bibliography: p.
Includes index.
1. Tissue culture. 2. Cell culture. I. Title.
[QH585.F74 1987] 591'.07'24 87-3313
ISBN 0-8451-4241-0

first by centrifugation 10,000 *g*, 20 min, or filtration through 5-μm and 1.2-μm filters) (see Chapter 9, section on sterilization of serum). (3) Add to cloning medium 1 part conditioned medium to 2 parts cloning medium.

Feeder layers (Fig. 11.3, regular feeder layer). (1) Trypsinize embryo fibroblasts from primary culture (see Chapters 9 and 10) and reseed at 10^5 cells/ml. (2) At 50% confluence, add mitomycin-C, 2μg/10^6 cells, 0.25 μg/ml, overnight [MacPherson and Bryden, 1971], or irradiate culture with 30 Gy (3,000 rad). (3) Change the medium after treatment, and after a further 24 hr, trypsinize the cells and reseed in fresh medium at 5×10^4 cells/ml (10^4 cells/cm^2). (4) Incubate for a further 24–48 hr and then seed cells for cloning. The feeder cells will remain viable for up to 3 wk but will eventually die out and are not carried over if the colonies are isolated.

Other cell lines or homologous cells may be used to improve the plating efficiency but heterologous cells have the advantage that if clones are to be isolated later, chromosome analysis will rule out accidental contamination from the feeder layer.

Hormones. Insulin, 1–10 IU/ml has been found to increase the plating efficiency of several cell types [Hamilton and Ham, 1977]. Dexamethasone, 2.5×10^{-5} M, ~10 μg/ml (a soluble synthetic hydrocortisone analogue) improves the plating efficiency of human normal glia, glioma, fibroblasts, and melanoma, and chick myoblasts, and will give increased clonal growth (colony size) if removed 5 d after plating [Freshney et al., 1980a,b]. Lower concentrations (10^{-7} M) have been found preferable for epithelial cells (see Chapter 20).

Intermediary metabolites. Keto acids, e.g., pyruvate or α-ketoglutarate, [Griffiths and Pirt, 1967; McKeehan and McKeehan, 1979] and nucleosides [α-medium, Stanners et al., 1971], have been used to supplement media and are already included in the formulation of a rich medium like Ham's F12. Pyruvate is also added to Dulbecco's modification of Eagle's MEM [Dulbecco and Freeman, 1959; Morton, 1970].

Carbon dioxide. CO_2 is essential to obtain maximum cloning efficiency for most cells. While 5% is most usual, 2% is sufficient for many cells, and may even be slightly better for human glia and fibroblasts. HEPES (20 mM) may be used with 2% CO_2, protecting the cells against pH fluctuations during feeding and in the event of failure of the CO_2 supply. (Using 2% CO_2 also cuts down in the consumption of CO_2.) At the other extreme, Dulbecco's modification of Eagle's MEM is normally equilibrated with 10% CO_2 and is frequently used for cloning myeloma hybrids for monoclonal antibody production. The concentration of bicarbonate must be adjusted if the CO_2 tension is altered so that equilibrium is reached at pH 7.4 (see Table 7.2).

Treatment of substrate. Polylysine improves the plating efficiency of human fibroblasts in low serum concentrations [McKeehan and Ham, 1976] (see Chapter 7). (1) Add 1 mg/ml poly-D-lysine in water to plates (~5 ml/25 cm^2). (2) Remove and wash plates with 5 ml PBSA per 25 cm^2. The plates may be used immediately or stored for several weeks before used.

Fibronectin also improves the plating of many cells [Barnes and Sato, 1980]. The plates should be pretreated with 5 μg/ml fibronectin incorporated in the medium.

Trypsin. Pure, twice recrystallized, trypsin used at 0.05 μg/ml may be preferable to crude trypsin, but there are conflicting reports on this. McKeehan [1977] noted a marked improvement in plating efficiency when trypsinization (pure trypsin) was carried out at 4°C.

Multiwell Dishes

If clones are to be isolated, cloning by dilution directly into microwells (microtitration dishes or 24-well plates, see Fig. 7.4) makes subsequent harvesting easier. The plates must be checked regularly after seeding, however, to confirm that either only one cell is present per well at the start or, if there is more than one cell per well, they are not clumped and that only one cell gives rise to a colony, i.e., that the colonies which form are truly clonal in origin, and only one colony forms in the well.

Semisolid Media

Some cells, particularly hemopoietic stem cells and virally transformed fibroblasts, will clone readily in suspension. To hold the colony together and prevent mixing, the cells are suspended in agar or methocel and plated out over an agar underlay or into nontissue culture grade dishes.

Cloning in agar. See Figure 11.4 and Chapters 15, 20, and 21.

Outline

Agar is liquid at high temperatures but is a gel at 36.5°C. Cells are suspended in warm agar, and,